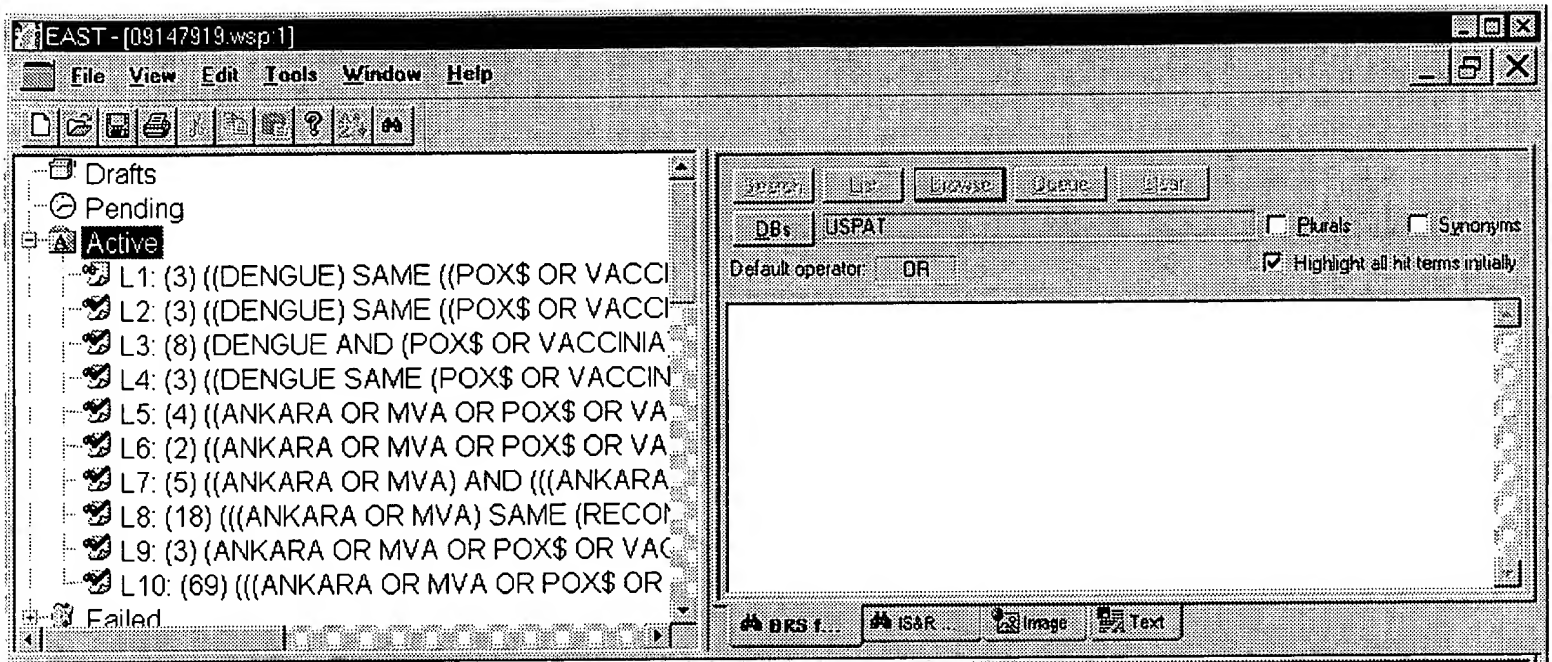

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3	BRS	L3	8	(DENGUE AND (POX$ OR VACCINIA)).CLM.	USPAT	2001/03/08 08:23	
4	BRS	L4	3	((DENGUE SAME (POX$ OR VACCINIA)) AND (ANKARA OR MVA))	USPAT	2001/03/08 08:23	
5	BRS	L5	4	((ANKARA OR MVA OR POX$ OR VACCINIA) SAME (SP6 OR "T7")) SAME (VACCINES OR VACCINE)	USPAT	2001/03/08 08:24	
6	BRS	L6	2	((ANKARA OR MVA OR POX$ OR VACCINIA) SAME (SP6 OR "T7")) SAME (administ$6)	USPAT	2001/03/08 08:25	
7	BRS	L7	5	((ANKARA OR MVA) AND (((ANKARA OR MVA OR POX$ OR VACCINIA) SAME (SP6 OR "T7")) and	USPAT	2001/03/08 08:25	
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9	BRS	L9	3	(ANKARA OR MVA OR POX$ OR VACCINIA) SAME (((SEROTYPES OR TYPES) SAME	USPAT	2001/03/08 08:28	
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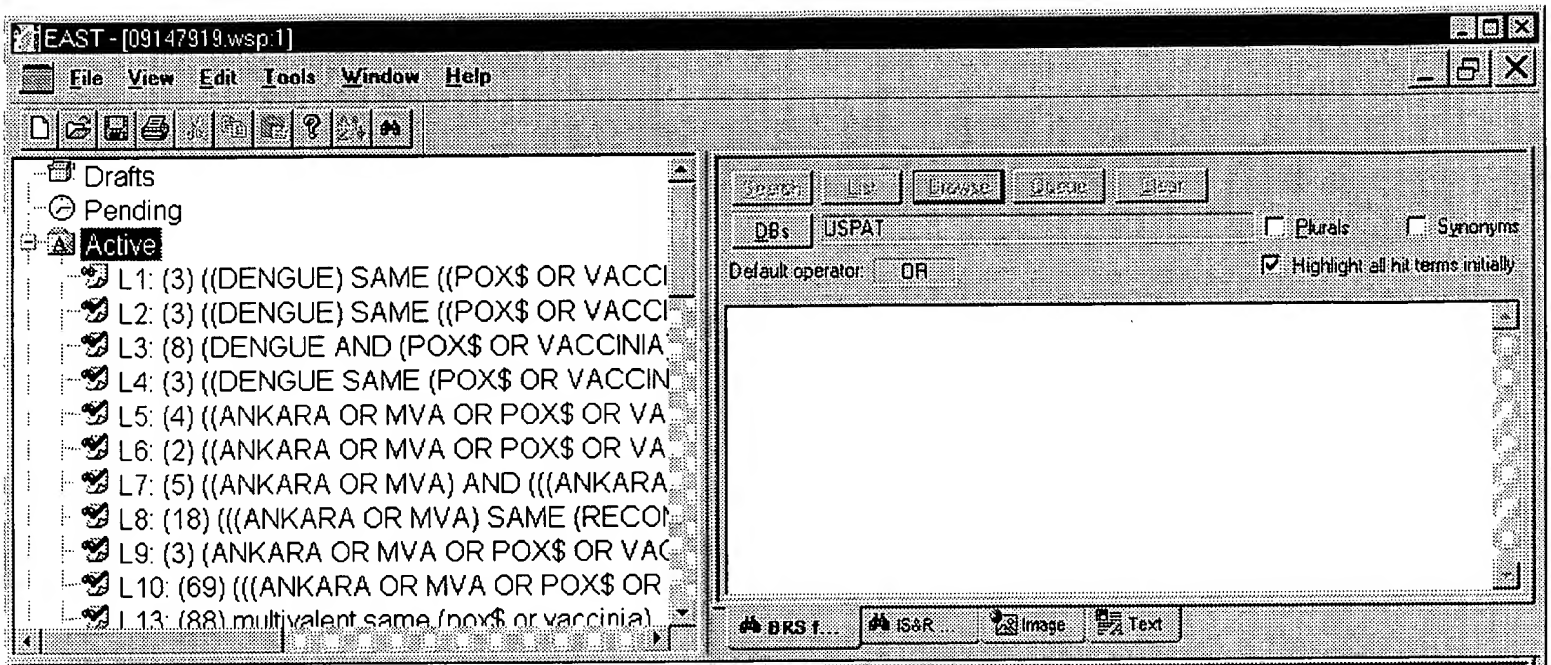

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3	BRS	L3	8	(DENGUE AND (POX$ OR VACCINIA)).CLM.	USPAT	2001/03/08 08:23	
4	BRS	L4	3	((DENGUE SAME (POX$ OR VACCINIA)) AND (ANKARA OR MVA))	USPAT	2001/03/08 08:23	
5	BRS	L5	4	((ANKARA OR MVA OR POX$ OR VACCINIA) SAME (SP6 OR "T7")) SAME (VACCINES OR VACCINE)	USPAT	2001/03/08 08:24	
6	BRS	L6	2	((ANKARA OR MVA OR POX$ OR VACCINIA) SAME (SP6 OR "T7")) SAME (administ$6)	USPAT	2001/03/08 08:25	
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8	BRS	L8	18	(((ANKARA OR MVA) SAME (RECOMBINANT OR VECTOR)) AND VACCINE$1)	USPAT	2001/03/08 08:26	
9	BRS	L9	3	(ANKARA OR MVA OR POX$ OR VACCINIA) SAME (((SEROTYPES OR TYPES) SAME	USPAT	2001/03/08 08:28	
10	BRS	L10	69	(((ANKARA OR MVA OR POX$ OR VACCINIA) SAME VACCINE$1) SAME (SEROTYPES OR TYPES))	USPAT	2001/03/08 08:28	
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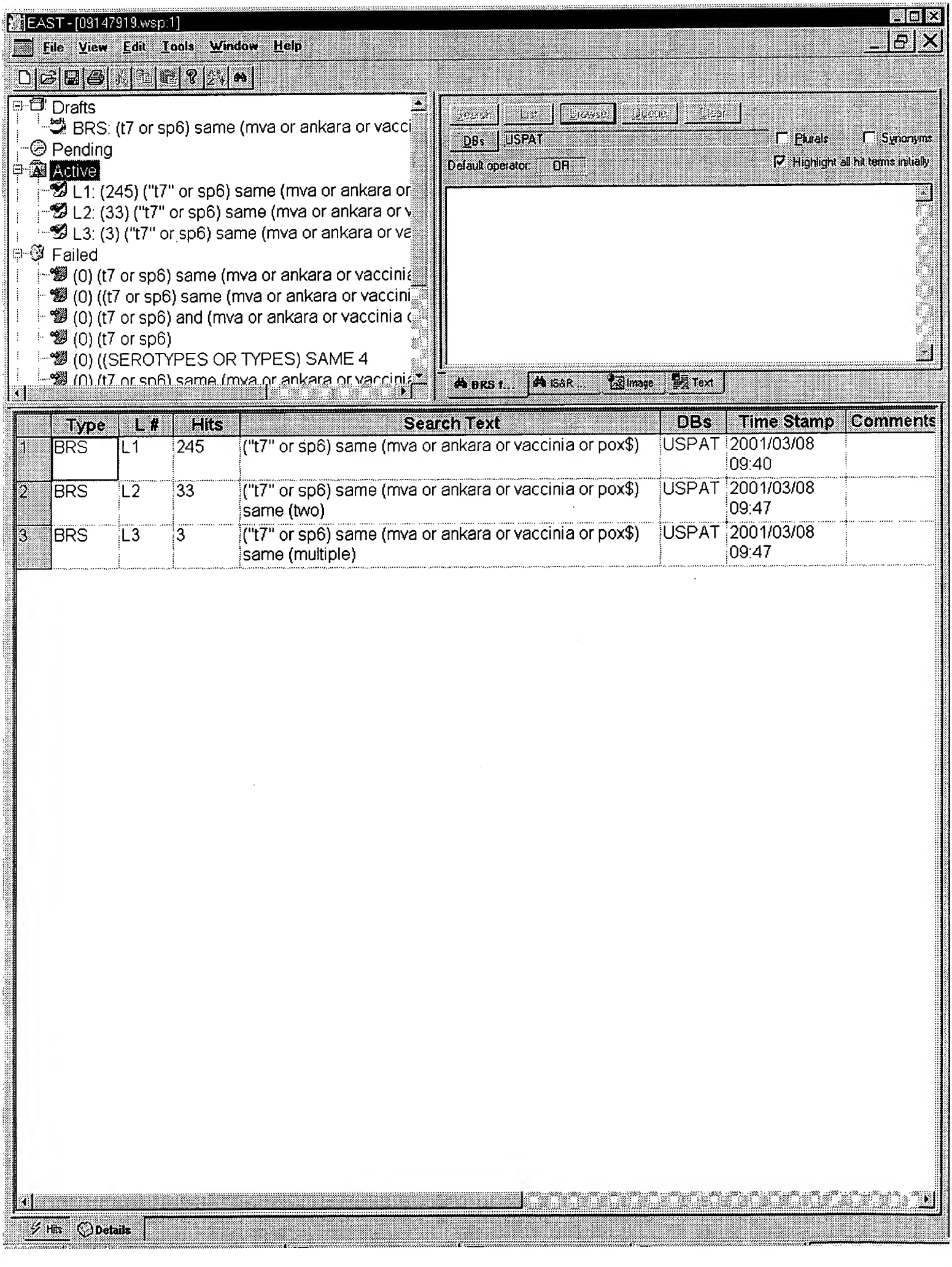
12	BRS	L14	40	multivalent with (pox$ or vaccinia)	USPAT	2001/03/08 08:41	
13	BRS	L15	12	multivalent same (pox$ or vaccinia) and serotypes	USPAT	2001/03/08 08:46	



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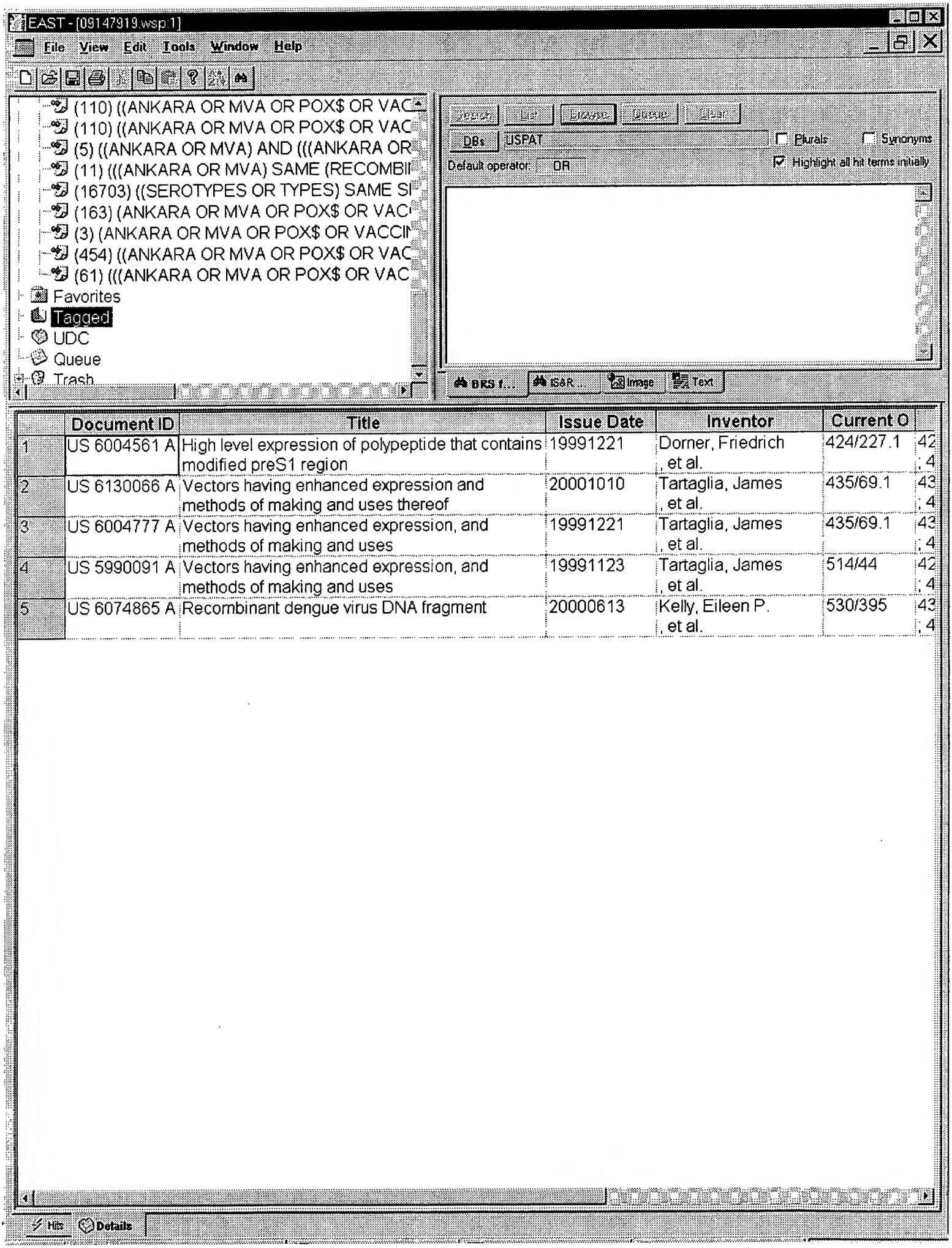
3	BRS	L3	3	("t7" or sp6) same (mva or ankara or vaccinia or pox$) same (multiple)	USPAT	2001/03/08 09:47	



	Document ID	Title	Issue Date	Inventor	Current O	
1	US 6004561 A	High level expression of polypeptide that contains modified preS1 region	19991221	Dorner, Friedrich , et al.	424/227.1	42 ; 4
2	US 6130066 A	Vectors having enhanced expression and methods of making and uses thereof	20001010	Tartaglia, James , et al.	435/69.1	43 ; 4
3	US 6004777 A	Vectors having enhanced expression, and methods of making and uses	19991221	Tartaglia, James , et al.	435/69.1	43 ; 4
4	US 5990091 A	Vectors having enhanced expression, and methods of making and uses	19991123	Tartaglia, James , et al.	514/44	42 ; 4
5	US 6074865 A	Recombinant dengue virus DNA fragment	20000613	Kelly, Eileen P. , et al.	530/395	43 ; 4

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*File 155: First Medline 2001 update is expected towards the end of March 2001. For other NLM information see Help News155.
File 357:Derwent Biotechnology Abs 1982-2001/Mar B2
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*File 357: Price changes as of 1/1/01. Please see HELP RATES 357.

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Set Items Description
S1 418 VACCINIA AND (SP6 OR T7)
S2 116 TWO AND S1
S3 115 RD (unique items)
S4 2 SIMULTANEOUS? AND S3
S5 19 S1 AND (TWO (3N)(PROTEINS OR PRODUCTS))
S6 36 MULTIPLE(W)PLASMIDS
S7 0 S6 AND S1
S8 116 PLASMIDS AND S1
S9 85 PY<1997 AND S8
S10 1 PLASMID(5N)GENES AND S1 AND PY<1997
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4/7/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2000 Dialog Corporation. All rts. reserv.
08667142 94120726
Expression of hepatitis A virus precursor protein P3 in vivo and in vitro: polyprotein processing of the 3CD cleavage site.
Tesar M; Pak I; Jia XY; Richards OC; Summers DF; Ehrenfeld E
Department of Molecular Biology and Biochemistry, University of California, Irvine 92717.
Virology (UNITED STATES) Feb 1994, 198 (2) p524-33, ISSN 0042-6822 Journal Code: XEA
Contract/Grant No.: AI-17386, AI, NIAID; AI-26350, AI, NIAID
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Hepatitis A virus (HAV) cDNAs encoding the P3 region proteins were expressed in vivo and in vitro to characterize the HAV 3D protein and to identify the cleavage site between 3C and 3D. Protein coding sequences were placed under control of a T7 promoter and an EMCV translational initiation signal. T7 RNA polymerase was provided by simultaneous infection of transfected BS-C-1 cells with a recombinant vaccinia virus vTF7-3 (T. R. Fuerst et al., Proc. Natl. Acad. Sci. USA 83, 8122-8126, 1986). Efficient synthesis and processing of P3 proteins occurred to yield 3CD (78 kDa), 3D (54 kDa), 3ABC (33 kDa), 3BC (25 kDa), and 3C (23 kDa). Similar products were produced by translation of T7 transcripts in a rabbit reticulocyte lysate in vitro. The 3C/D cleavage site was mapped by comparing the mobility of 3D in SDS-PAGE with 3D proteins engineered to begin at each of the two proposed cleavage sites; in addition, direct N-terminal sequencing of radiolabeled 3D protein from translation in vitro was performed. The results showed that 3D was formed by cleavage at the glutamine-arginine (Q/R) pair at position 1738 and 1739 of the HAV polyprotein. HAV 3D protein produced by autocatalytic cleavage of P3 precursor proteins in BS-C-1 cells is virtually completely insoluble and sediments after low-speed centrifugation. This is in contrast to the poliovirus 3D protein, produced from a similar construct, a significant portion of which remains soluble. Extracts containing the poliovirus 3D protein manifested high levels of RNA-dependent RNA polymerase activity, whereas those containing the HAV 3D protein showed no detectable activity by the same assay.

4/7/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2000 Dialog Corporation. All rts. reserv.
07620999 93388562
The DNA-dependent ATPase activity of vaccinia virus early gene transcription factor is essential for its transcription activation function.
Li J; Broyles SS
Department of Biochemistry, Purdue University, West Lafayette, Indiana 47907-1153.
Journal of biological chemistry (UNITED STATES) Sep 25 1993, 268 (27) p20016-21, ISSN 0021-9258 Journal Code: HIV
Contract/Grant No.: AI 28432-01, AI, NIAID
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Vaccinia virus early transcription factor (VETF) activates the transcription of early gene templates by the viral RNA polymerase. VETF is a heterodimeric protein that binds to transcription promoters and has an associated DNA-dependent ATPase activity. The small subunit of VETF has sequences resembling two motifs commonly found in ATPases: an A-type ATP binding motif and a DEAH box. To investigate the functional role of the ATPase activity, we have analyzed the effect of mutations in each of the

putative ATPase motifs. Recombinant VETF was expressed in HeLa cells using a vaccinia virus/T7 RNA polymerase system. Simultaneous expression of both subunits of VETF was required to obtain soluble protein with promoter binding, DNA-dependent ATPase, and transcription activation functions. The mutants with altered ATPase motifs retained promoter binding activity but had no detectable ATPase activity and no ability to activate transcription. The DEAH box mutant was shown to dominantly repress transcription activation by wildtype VETF. These results indicate that the DNA-dependent ATPase activity of VETF is essential for its transcription activation function.

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$5.06 Estimated total session cost 1.123 DialUnits
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9/7/28 (Item 28 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08218951 95124243
Use of recombinant vaccinia virus vectors for cell biology.
Weisz OA; Machamer CE
Department of Cell Biology and Anatomy, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.
Methods in cell biology (UNITED STATES) 1994, 43 Pt A p137-59, ISSN 0091-679X Journal Code: MV4
Languages: ENGLISH
Document type: JOURNAL ARTICLE; REVIEW; REVIEW, TUTORIAL
(21 Refs.)

9/7/29 (Item 29 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08207858 94322007
Synthesis of biologically active influenza virus core proteins using a vaccinia virus-T7 RNA polymerase expression system.
Mena I; de la Luna S; Albo C; Martin J; Nieto A; Ortin J; Portela A
Centro Nacional de Microbiologia Virologia e Inmunologia Sanitarias, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain.
Journal of general virology (ENGLAND) Aug 1994, 75 (Pt 8) p2109-14, ISSN 0022-1317 Journal Code: I9B
Languages: ENGLISH
Document type: JOURNAL ARTICLE
An in vivo system in which expression of a synthetic influenza virus-like chloramphenicol acetyltransferase (CAT) RNA is driven by influenza virus proteins synthesized from cloned cDNAs has been developed. Expression of the four influenza virus core proteins (nucleoprotein, PA, PB1 and PB2) was performed by transfection of four pGEM recombinant plasmids, each containing one of the four viral genes, into cell cultures previously infected with a vaccinia virus recombinant encoding the T7 RNA polymerase (vTF7-3). When a naked negative-sense influenza virus-like CAT RNA was transfected into cells expressing the four influenza virus proteins, CAT activity was detected in the cell extracts, demonstrating that the expressed proteins had RNA-synthesizing activity. In this system, CAT RNA templates containing additional nucleotides at the 3' end were also expressed, resulting in CAT activity. This showed that the influenza virus polymerase can recognize its promoter when located internally on an RNA template. In influenza virus-infected cells however, CAT activity was detected only when the CAT RNA contained the viral promoter at the exact 3' end and was transfected as in vitro assembled ribonucleoprotein. These results are discussed in terms of the different requirements of the two helper systems for expression of an exogenously added RNA.

9/7/30 (Item 30 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08201351 94220164
Expression of functional influenza virus A polymerase proteins and template from cloned cDNAS in recombinant vaccinia virus infected cells.
Zhang H; Air GM
Department of Microbiology, University of Alabama at Birmingham 35294.
Biochemical and biophysical research communications (UNITED STATES) Apr 15 1994, 200 (1) p95-101, ISSN 0006-291X Journal Code: 9Y8
Contract/Grant No.: AI 19084, AI, NIAID; AI 18203, AI, NIAID
Languages: ENGLISH
Document type: JOURNAL ARTICLE
cDNAs containing the coding sequences of influenza type A virus polymerase proteins (PB1, PB2 and PA) and nucleoprotein (NP) have been expressed in mammalian cells by T7 polymerase provided by a recombinant vaccinia virus. The resulting proteins are able to form a complex that can copy a negative sense influenza-like RNA, transcribed from input DNA by the T7 polymerase, into a positive sense RNA that is translated into active chloramphenicol acetyltransferase (CAT). In this system there is no requirement for helper virus or purified viral core proteins, thus it will

allow manipulation of all proteins as well as template for studies of replication in influenza virus.

9/7/39 (Item 39 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07811747 93159781
SP6 RNA polymerase containing vaccinia virus for rapid expression of cloned genes in tissue culture.
Usdin TB; Brownstein MJ; Moss B; Isaacs SN
Natl. Institute of Mental Health, National Institutes of Health, Bethesda, MD 20892.
BioTechniques (UNITED STATES) Feb 1993, 14 (2) p222-4, ISSN 0736-6205 Journal Code: AN3
Languages: ENGLISH
Document type: JOURNAL ARTICLE
A hybrid transient expression system, in which tissue culture cells are infected with a recombinant vaccinia virus encoding bacteriophage DNA-dependent RNA polymerase and transfected with a plasmid containing a cloned gene behind the bacteriophage promoter, allows rapid high-level expression in nearly 100% of the cells. In order to extend this system to clones from libraries containing SP6 promoters, a new vaccinia virus was constructed encoding bacteriophage SP6 RNA polymerase.

9/7/47 (Item 47 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07404028 93119730
A plasmid that improves the efficiency of foreign gene expression by intracellular T7 RNA polymerase.
Takahashi T; Ryan KW; Portner A
Department of Virology and Molecular Biology, St. Jude Children's Research Hospital, Memphis, Tennessee 38101-0318.
Genetic analysis, techniques and applications (UNITED STATES) Jun 1992, 9 (3) p91-5, ISSN 1050-3862 Journal Code: AP4
Contract/Grant No.: AI 11949, AI, NIAID; AI 31596, AI, NIAID; CA 21765, CA, NCI
Languages: ENGLISH
Document type: JOURNAL ARTICLE
To facilitate the construction of recombinant plasmids for expressing cloned genes with T7 RNA polymerase supplied by recombinant vaccinia virus, a plasmid expression vector was designed by combining parts of plasmids pTZ18R, pBluescript II KS+, and pAR2529. The 3043-bp plasmid pTF1 has a T7 RNA polymerase promoter, multiple cloning site for insertion of foreign genes, and a T7-specific transcription termination signal. Plasmid pTF1 had several advantages compared with the reference plasmid pAR2529, including more efficient replication in bacteria, greater flexibility in the insertion and subcloning of foreign genes, and increased efficiency of liposome-mediated introduction into cultured cells for expression of the foreign gene.

9/7/52 (Item 52 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07366596 91321052
Vaccinia-T7 RNA polymerase expression system: evaluation for the expression cloning of plasma membrane transporters.
Blakely RD; Clark JA; Rudnick G; Amara SG
Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, Connecticut 06510.
Analytical biochemistry (UNITED STATES) May 1 1991, 194 (2) p302-8, ISSN 0003-2697 Journal Code: 4NK
Contract/Grant No.: 5 T32 GM07324-14, GM, NIGMS
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The vaccinia/T7 transient expression system, which results in rapid, high-level expression of proteins encoded by plasmids bearing T7 promoters, provides a powerful strategy for the expression cloning of membrane transporters. To test the feasibility of this approach, we introduced the rabbit Na+/glucose transporter by liposome-mediated transfection into vaccinia infected HeLa cells and determined the characteristics and sensitivity of induced [14C]alpha-methyl D-glucopyranoside uptake. We observed a rapid (4-12 h) expression of saturable (Kt = 342 microM) [14C]alpha-methyl D-glucopyranoside uptake following transfection, with substrate and inhibitor sensitivities of the native carrier, including Na+ and temperature dependence and appropriate phloridzin sensitivity (KI = 9.1 microM). The time-dependent increase in alpha-methyl D-glucopyranoside uptake coincided with a decline in endogenous Na+/D-aspartate transport. Maximal levels of expression achieved were nearly 10-fold higher than that reported for transient expression of Na+/glucose transporters in the COS cell system. Rate and dilution estimates demonstrates a sensitivity of detection of single clones diluted several thousand fold by nonspecific plasmid DNA. A further 3-fold increase in transport sensitivity was achieved after transfection of plasmid constructs bearing additional 5'-T7 stem-loop and 3'-T7 termination signals. When cell lines with low endogenous transport were coupled with substrates of high specific activity, as with measurements of induced [3H]gamma-aminobutyric acid uptake, we were able to detect expression from transporter bearing plasmids diluted as much as 10,000-fold by non-specific plasmid DNA.(ABSTRACT TRUNCATED AT 250 WORDS)

9/7/61 (Item 61 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06915018 91142176
Cells that express all five proteins of vesicular stomatitis virus from cloned cDNAs support replication, assembly, and budding of defective interfering particles.
Pattnaik AK; Wertz GW
Department of Microbiology, University of Alabama, Birmingham Medical School 35294.
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Feb 15 1991, 88 (4) p1379-83, ISSN 0027-8424 Journal Code: PV3
Contract/Grant No.: AI 20181, AI, NIAID; R37 AI 12464, AI, NIAID
Languages: ENGLISH
Document type: JOURNAL ARTICLE
An alternative approach to structure-function analysis of vesicular stomatitis virus (VSV) gene products and their interactions with one another during each phase of the viral life cycle is described. We showed previously by using the vaccinia virus-T7 RNA polymerase expression system that when cells expressing the nucleocapsid protein (N), the phosphoprotein (NS), and the large polymerase protein (L) of VSV were superinfected with defective interfering (DI) particles, rapid and efficient replication and amplification of (DI) particle RNA occurred. Here, we demonstrate that all five VSV proteins can be expressed simultaneously when cells are contransfected with plasmids containing the matrix protein (M) gene and the glycoprotein (G) gene of VSV in addition to plasmids containing the genes for the N, NS, and L proteins. When cells coexpressing all five VSV proteins were superinfected with DI particles, which because of their defectiveness are unable to express any viral proteins or to replicate, DI particle replication, assembly, and budding were observed and infectious DI particles were released into the culture fluids. Omission of either the M or G protein expression resulted in no DI particle budding. The vector-supported DI particles were similar in size and morphology to the authentic DI particles generated from cells coinfected with DI particles and helper VSV and their infectivity could be blocked by anti-VSV or anti-G antiserum. The successful replication, assembly, and budding of DI particles from cells expressing all five VSV proteins from cloned cDNAs provide a powerful approach for detailed structure-function analysis of the VSV gene products in each step of the replicative cycle of the virus.

9/7/64 (Item 64 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2000 Dialog Corporation. All rts. reserv.
06846081 92112061
A new vaccinia recombinant plasmid pSC-O.
Scheiflinger F; Bodemer W
IMMUNO AG., Biomedical Research Center, Orth a.d. Donau, Austria.
Gene (NETHERLANDS) Dec 30 1991, 109 (2) p307-8, ISSN 0378-1119 Journal Code: FOP
Languages: ENGLISH
Document type: JOURNAL ARTICLE
A new plasmid pSC-O for generation of recombinant vaccinia virus was constructed. It offers additional advantages when compared with other widely used insertion vectors. This plasmid allows the expression of coding sequences lacking codons for the initiation as well as for termination of translation. Additional sequences modulating translation, but also transcription or affecting intracellular processing can be introduced. Sequences flanking the transcription unit of the gene of interest are complementary to SP6/T7 sequencing primers and thus may allow rapid sequencing (amplification) of the inserted DNA.

9/7/69 (Item 69 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2000 Dialog Corporation. All rts. reserv.
06186376 87041412
Eukaryotic transient-expression system based on recombinant vaccinia virus that synthesizes bacteriophage T7 RNA polymerase.
Fuerst TR; Niles EG; Studier FW; Moss B
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Nov 1986, 83 (21) p8122-6, ISSN 0027-8424 Journal Code: PV3
Languages: ENGLISH
Document type: JOURNAL ARTICLE
DNA coding for bacteriophage T7 RNA polymerase was ligated to a vaccinia virus transcriptional promoter and integrated within the vaccinia virus genome. The recombinant vaccinia virus retained infectivity and stably expressed T7 RNA polymerase in mammalian cells. Target genes were constructed by inserting DNA segments that code for beta-galactosidase or chloramphenicol acetyltransferase into a plasmid with bacteriophage T7 promoter and terminator regions. When cells were infected with the recombinant vaccinia virus and transfected with plasmids containing the target genes, the latter were expressed at high levels. Chloramphenicol acetyltransferase activity was 400-600 times greater than that observed with conventional mammalian transient-expression systems regulated either by the enhancer and promoter regions of the Rous sarcoma virus long terminal repeat or by the simian virus 40 early region. The vaccinia/T7 hybrid virus forms the basis of a simple, rapid, widely applicable, and efficient mammalian expression system.

9/7/79 (Item 6 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs
(c) 2001 Derwent Publ Ltd. All rts. reserv.
0156412 DBA Accession No.: 93-14464
Vaccinia virus and vaccinia virus/bacteriophage T7 hybrid expression vectors - vaccinia virus-phage T7 hybrid vector construction; potential use e.g. as recombinant vaccine (conference paper)

AUTHOR: Moss B; Fuerst T R
CORPORATE SOURCE: Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892, USA.
JOURNAL: Viral Vectors (85-90) 1988
CODEN: 9999R
LANGUAGE: English
ABSTRACT: The molecular biology of vaccinia virus (VV) and the construction of VV expression vectors were summarized. An alternative expression system was developed that utilized the highly efficient and selective phage T7 RNA-polymerase (EC-2.7.7.6) in mammalian cells. The single subunit phage polymerase gene was placed next to a VV promoter and incorporated into VV. Cells infected with the recombinant virus expressed T7 RNA-polymerase. By transfecting plasmids containing foreign genes with T7 promoter and termination sequences into these cells, specific protein expression was obtained. Higher expression occurred if the foreign gene with T7 regulatory sequences was recombined into VV. However, separate viruses were required for T7 RNA-polymerase and T7 promoter because their combination in a single virus was lethal. About 30% of the total cell RNA present 24-48 hr after coinfection consisted of T7 promoter transcripts. The RNA contained the correct 5' end but only 5-10% was capped. Expressed protein reached 3% of total cell protein. The VV and hybrid VV/T7 vectors may be useful for the development of subunit and live vaccines. (26 ref)

9/7/80 (Item 7 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs

0145046 DBA Accession No.: 93-03098
SP6 RNA-polymerase containing vaccinia virus for rapid expression of cloned genes in tissue culture - gene cloning using vaccinia virus vSIMB7.5 or vSIMBE/L vector with phage SP6 RNA-polymerase gene in e.g. CV-1 cell culture
AUTHOR: Usdin T B; Brownstein M J; +Moss B; Isaacs S N
CORPORATE SOURCE: Laboratory of Viral Diseases, Bldg. 4, Rm. 229, National Institute of Allergy and Infectious Diseases, NIH, 9000 Rockville Pike, Bethesda, MD 20892, USA.
JOURNAL: BioTechniques (14, 2, 222-24) 1993
CODEN: BTNQDO
LANGUAGE: English
ABSTRACT: A hybrid transient expression system, in which cell cultures were infected with a recombinant vaccinia virus (VV) vector encoding phage RNA-polymerase (RP, EC-2.7.7.6) and transfected with a plasmid containing a cloned gene behind a phage promoter (giving rapid high-level expression) was extended to clones from gene banks with phage SP6 promoters. 2 New VV vectors were constructed, encoding phage SP6 RP by recombination of homologous sequences in plasmids and wild-type VV DNA using an infection-transfection protocol. The resulting viruses, vSIMB7.5 and vSIMBE/L, expressed the SP6 RP gene under the control of the VV P7.5 promoter and a synthetic early/late promoter, respectively. An SP6 promoter-regulated beta-galactosidase (BG, EC-3.2.1.23) reporter plasmid was used as a secondary screen for recombinants expressing functional RP. The utility of the system was demonstrated by transfection of a CV-1 cell culture. Nearly 100% of transfected cells were recombinant. A major advantage of these methods was that high levels of transient expression were obtained after less than 24 hr. (13 ref)

9/7/81 (Item 8 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs

0143226 DBA Accession No.: 93-01278
A plasmid that improves the efficiency of foreign gene expression by intracellular T7 RNA-polymerase - recombinant plasmid pTF1 for improved foreign gene expression
AUTHOR: Takahashi T; Ryan K W; +Portner A
CORPORATE SOURCE: Department of Virology and Molecular Biology, St. Jude Children's Research Hospital, 332 North Lauderdale, Memphis TN 38101-0318, USA.
JOURNAL: Genet.Anal.Tech.Appl. (9, 3, 91-95) 1992
CODEN: 4353H
LANGUAGE: English
ABSTRACT: To facilitate the construction of recombinant plasmids for expressing cloned genes with phage T7 RNA-polymerase (EC-2.7.7.6) supplied by recombinant vaccinia virus, a plasmid expression vector was designed by combining parts of plasmid pTZ18R, plasmid pBluescript-IIKS+ and plasmid pAR2529. The 3043-bp plasmid pTF1 had a phage T7 RNA-polymerase promoter, multiple cloning site for insertion of foreign genes, and a T7-specific transcription termination signal. pTF1 had several advantages compared with the reference plasmid pAR2529, including more efficient replication in bacteria, greater flexibility in the insertion and subcloning of foreign genes, and increased efficiency of liposome-mediated introduction into cultured cells for expression of the foreign gene. Plasmid pTF1 offers significant technical advantages in T7 RNA-polymerase-mediated intracellular gene expression. In addition to pTF1, several commercially available plasmid vectors designed for in vitro transcription by T7 RNA-polymerase have been used successfully in vaccinia-T7 intracellular expression systems. (11 ref)

9/7/85 (Item 12 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs

0056852 DBA Accession No.: 87-01200
Eukaryotic transient-expression system based on recombinant vaccinia virus that synthesizes bacteriophage T7 RNA-polymerase - vector construction
AUTHOR: Fuerst T R; Niles E G; Studier F W; +Moss B
CORPORATE SOURCE: Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA.
JOURNAL: Proc.Natl.Acad.Sci.U.S.A. (83, 21, 8122-26) 1986
CODEN: PNASA6
LANGUAGE: English
ABSTRACT: DNA coding for phage T7 RNA-polymerase (EC-2.7.7.6) was ligated to a vaccinia virus transcriptional promoter and integrated within the vaccinia virus genome. The recombinant vaccinia virus retained infectivity and stably expressed T7 RNA-polymerase in mammalian cells. Target genes were constructed by inserting DNA segments that code for beta-galactosidase (EC-3.2.1.23) or chloramphenicol-acetyltransferase (EC-2.3.1.28) into a plasmid with phage T7 promoter and terminator regions. When cells were infected with the recombinant vaccinia virus and transfected with plasmids containing the target genes, the latter were expressed at high levels. Chloramphenicol-acetyltransferase activity was 400-600 times greater than that observed with conventional mammalian transient-expression systems regulated either by the enhancer and promoter regions of the Rous-sarcoma virus long terminal repeat or by the SV40 early region. The vaccinia/T7 hybrid virus forms the basis of a simple, rapid, widely applicable, and efficient mammalian expression system. (25 ref)
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10/7/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

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Analysis of respiratory syncytial virus F, G, and SH proteins in cell fusion.
Heminway BR; Yu Y; Tanaka Y; Perrine KG; Gustafson E; Bernstein JM; Galinski MS
Department of Molecular Biology, Cleveland Clinic Foundation, Ohio 44195.
Virology (UNITED STATES) May 1 1994, 200 (2) p801-5, ISSN 0042-6822 Journal Code: XEA
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Recombinant expression of the human respiratory syncytial virus (RSV) fusion (F) glycoprotein, receptor-binding glycoprotein (G), and small hydrophobic (SH) protein was performed to determine the role(s) of these proteins in syncytia formation. These studies used a vaccinia virus expressing the bacteriophage (T7) RNA polymerase gene and plasmid vectors containing the RSV genes under the control of a T7 promoter. Within the context of this expression system, expression of any individual RSV gene, or coexpression of F+G genes, did not elicit the formation of syncytia. However, at plasmid input levels which were 10-fold higher than those normally used, coexpression of F+G induced low but detectable levels of cell fusion. In contrast, coexpression of F, G, and SH together elicited extensive cell fusion resembling that of an authentically infected cell monolayer. In addition, coexpression of F and SH elicited significant cell fusion, although to a lesser extent than was observed when G was included. Cell fusion induced by coexpression of F+SH was found to be specific to the RSV proteins, since coexpression of SH with the analogous F proteins from human parainfluenza virus type 3, human parainfluenza virus type 2, Sendai virus, or simian virus type 5 (SV5) did not elicit cell fusion. Finally, coexpression of the SV5 SH protein with the RSV or SV5 glycoproteins also failed to induce syncytia, suggesting type-specific restrictions between the two sets of viral proteins.
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